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Original Research Article

Genotyping and antifungal susceptibility profile of *Candida albicans* isolated from various clinical samples from tertiary care hospital

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ABSTRACT

Background: Genotyping *Candida albicans* helps identify outbreaks, sources, and virulent and drug-resistant strains. PCR can identify *Candida albicans* isolates as A, B, or C based on transposable group I introns in 25S rDNA. The length of the amplified product divides *C. albicans* isolates into genotypes A, B, and C. Objective of this study was to determine *Candida albicans* genotypes and antifungal susceptibility in candidiasis patients using PCR.
Methods: HIV-positive vaginal and oropharyngeal swabs yielded 126 *Candida albicans* isolates. Germ tube development, chlamydospore formation, CHROM agar color, sugar fermentation, and auxanographic plate technique with sugar discs identified all *C. albicans* isolates phenotypically. E-test tested antifungal susceptibility. Genotyping was done using PCR. The Phenol: Chloroform method extracted genomic DNA from all C. albicans isolates. The primer pairs and sequence encompassed the 25Sr DNA transposable intron. 5'-ATA AGG GAA GTC GGC AAA GAT CCG TAA-3' and CA-INT-R (reverse): 5'-CCT TGG CTG TGG TTT CGC TAG ATA GTA GAT-3'.
Results: Out of all the 126 profiles, PCR examination of the 25S rDNA transposable group I intron indicated 68.3% genotype A, 10.3% genotype B, and 21.4% genotype C isolates. Genotype A had 16.3% fluconazole resistance, genotype B 7.7%, and genotype C 3.7%. Genotype A strains were resistant to caspofungin, amphotericin B, flucytosine, and voriconazole at 3.5%, 5.8%, 8.1%, and 8.2%. While genotype B and C strains were sensitive.
Conclusions: *C. albicans* genotyping advances drug resistance research. *C. albicans* molecular typing helps design infection control methods by studying its epidemiology and pathophysiology.

Keywords: Candida albicans, Genotypes, Antifungal resistance, PCR

INTRODUCTION

Fungal diseases are increased and gained clinical importance in the second half of the last century, primarily due to improvements in medical technologies such as advent of immunosuppressive and transplantation therapies, the AIDS pandemic and increased use of antibiotics. This has resulted in increase in the incidence of fungal infections, mainly those due to *Candida* species. *Candida* species are emerged as main pathogens in both invasive and mucosal infections.[1,2] Non-*albicans Candida* species have been increasingly documented in the past 2-3 decades concurring with increase in the usage of azole antifungals particularly fluconazole.[3-5] Among *Candida* species *Candida albicans* remains to be the most commonly isolated species from clinical specimens. Emergence of *C. albicans* strains resistant to antifungal drugs such as fluconazole is now a documented problem.[6,7] Hence, knowledge of resistance to conventional antifungals and investigation of newer antifungal agents could support in better disease management.[6,7] The epidemiology of *Candida albicans* infection is complex.

Previous authors relied on not-reproducible and insensitive tests measuring a variety of phenotypic properties.[8] Powerful DNA-based typing tools such as pulsed field gel electrophoresis, restriction endonuclease analysis and multilocus sequence typing have emerged as the new tools for epidemiological studies and have been used for *C. albicans* strain differentiation. Several authors have compared diverse genotyping methods for *C. albicans*.[8,9] Using ribosomal sequences designed for genetic typing has also been used widely for typing fungal pathogens. A PCR based method with primers designed to span the region that includes the site of the transposable group I intron of the 25S rRNA gene (rDNA) developed by McCullough et al has been shown to be useful in classifying *C. albicans* into 5 genotypes A–E. Genotype D and E are found to fit to the same taxon as *C. dubliniensis*, a of late described yeast phenotypically analogous to *C. albicans*.[9,10] 25S rDNA PCR has been shown to be an easy to do method in providing clear and consistent results and thus could be used for analysis of a large number of isolates.[6,7] With no complete data available on drug resistance profile and genotypes of *C. albicans* from this region, the present study was planned as a preliminary investigation on genotype distribution of *C. albicans* isolates from candidiasis cases from Davangere, Karnataka and to determine the antifungal susceptibility of the isolates to five different antifungal agents including the newer agents – voriconazole and caspofungin.

METHODS

Isolation and phenotypic identification

This original research study was done between September 2023 to December 2024 which investigated a total of 126 *Candida albicans* isolated from HIV positive candidiasis cases from J. J. M. Medical college Davangere. The isolates were recovered from vaginal and oropharyngeal swabs of HIV positive cases. Most of the isolates were from high vaginal swabs (HVS) of female patients with vaginal candidiasis. The phenotypic identification of all *Candida albicans isolates* was confirmed by germ tube growth test, chlamydospore formation, color on CHRO Magar (CHROM agar HI-Media Mumbai), sugar fermentation and assimilation test by the auxanographic plate method using sugar discs (Glucose, sucrose, lactose, maltose, xylose, cellobiose galactose, arabinose, raffinose, rhamnose, trehalose). Single colony of all the isolates were selected from Sabouraud agar plates (SDA), and incubated at 37°C for 48 hours and then stored at 4°C before DNA extraction.[1,2] The Antifungal susceptibility to amphotericin B, fluconazole, voriconazole, flucytosine and caspofungin were performed by E – Test according to Mokaddas et al in RPMI medium.[5]

Inclusion criteria

The study included clinical isolates of *Candida albicans* obtained from high vaginal and oropharyngeal swabs of HIV-positive patients irrespective of age and sex presenting with candidiasis at J.J.M. Medical College, Davangere, during the study period. Only isolates that were phenotypically confirmed as *C. albicans* were considered.

Exclusion criteria

Isolates identified as non-*albicans Candida* species or derived from clinical specimens other than vaginal and oropharyngeal swabs were excluded. Additionally, duplicate isolates from the same patient and samples showing contamination with bacterial or fungal flora other than *Candida* were excluded from the analysis.

Genotypic identification by polymerase chain reaction (PCR)

DNA extraction - genomic DNA from all *C. albicans* isolates was extracted by Phenol: Chloroform method as described by Bii et al.[11] The PCR amplification was done as described by Zho et al. with modification. All *C. albicans* isolates were genotyped by amplifying exact DNA fragment located in the 25S rDNA bearing the potential Group I introns.[7] According to the condition, the PCR fragments of the strains with a band of 450bp were designated as genotype A, with a band of 840bp as genotype B, and with bands of both 450 and 840 bp as genotype C. The primer pairs and sequence used were as described by Zho et al spanning the site of the transposable intron in the 25Sr DNA. CA-INT-L (forward): 5'-ATA AGG GAA GTC GGC AAA ATA GAT CCG TAA-3' and CA-INT-R (reverse): 5'- CCT TGG CTG TGG TTT CGC TAG ATA GTA GAT-3'.[7] PCR was performed in an automated thermal cycler. Amplification reactions were performed in 25 µl reaction volumes and each PCR vial contained 1 µl genomic DNA, 2.5 µl 10 × PCR buffer, 2.5 µl $MgSO_4$, 0.5 µl dNTPs (10 mM), 1 µl forward primer (10 µM) 1 µl reverse primer (10 µM), 0.2 µl Taq DNA polymerase (2.5 U/ml), and 16.3 µl nuclease free water. Mixed to bring down the contents and placed into PCR thermal cycler. Amplification was performed fallowing PCR parameters, 96^0C for 5 min for initial denaturation, followed by 35 cycles of 94^0C for 30 sec for denaturation, 55^0C for 30 sec for annealing and 72^0C for 30 sec for extension, followed by an extension step at 72^0C 10 min. An aliquot of 5 µl of the PCR product was analyzed with 1% agarose gel to visualize the amplicons after staining with ethidium bromide.

RESULTS

In the present study distribution of *C. albicans* genotypes and antifungal susceptibility pattern were investigated. A total of 126 *C. albicans* isolates from high vaginal and oropharyngeal samples were characterized by 25s rDNA PCR into three genotypes. According to the criterion the strains with only one band of 450bp were designated as genotype A, with one band of 840bp as genotype B, and with bands of both 450bp and 840bp as genotype C. All the clinical isolates could be separated into three

genotypes, and other DNA types were not observed in this study. Genotypic analysis of *C. albicans* isolates indicated that 68.3% belonged to genotype A, the second common genotype was C 21.4% followed by 10.3% of genotype B (Table 1 and Figure 1). Resistance to fluconazole was observed in 16.3% of genotype A, 7.7% of genotype B, and 3.7% of genotype C. Resistance rate of 3.5%, 5.8%, 8.1% and 8.2% was showed by genotype A strains to caspofungin, amphotericin B, flucytosine and voriconazole respectively. Whereas all, genotype B and genotype C strains were sensitive to these four antifungals (Table 2).

Table 1: Distribution of *C. albicans* genotypes.

Sample	Total No	Genotype A	Genotype B	Genotype C
HVS	73	51	7	15
ORS	53	35	6	12
Total	126	86	13	27

HVS=High vaginal swabs, ORS= Oropharyngeal swabs

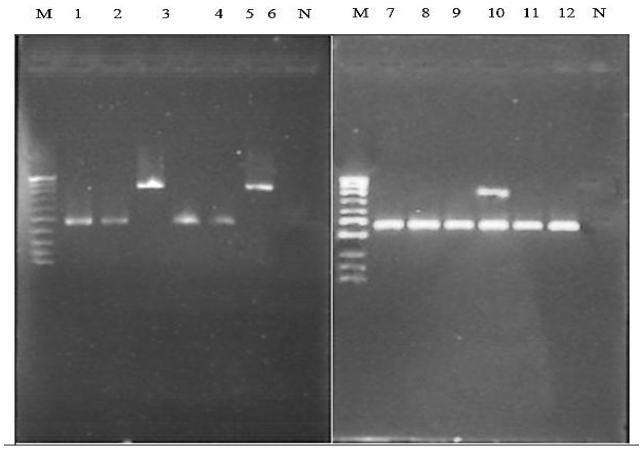

Figure 1: PCR gel image of DNA band patterns of *C. albicans* genotypes.

Lane M = DNA ladder 1000 kb, Lane N = Nuclease free water, Lane 1,2,4,5 = Genotype A (450bp), Lane 3, 6 = Genotype B (840bp), Lane 7,8,9,11,12 = Genotype A (450bp), Lane 10 = Genotype C (450 & 840bp).

Table 2: Antifungal susceptibility test of *C. albicans* genotypes.

Antifungal susceptibility pattern		Genotype A 86	Genotype B 13	Genotype C 27
		N (%)	**N (%)**	**N (%)**
Amphotericin B	S	81 (94.2)	13 (100)	27 (100)
	R	5 (5.8)	0	0
Fluconazole	S	67 (77.9)	12 (92.3)	26 (96.3)
	R	14 (16.3)	1 (7.7)	1 (3.7)
	SDD	5 (5.8)	0	0
Voriconazole	S	75 (88.4)	13 (100)	27 (100)
	R	8 (8.2)	0	0
	SDD	3 (3.4)	0	0
Flucytosine	S	79 (91.9)	13 (100)	27 (100)
	R	7 (8.1)	0	0
	SDD	0	0	0
Caspofungin	S	83 (96.5)	13 (100)	27 (100)
	R	3 (3.5)	0	0

S= Sensitive, R=Resistant, SDD= Susceptible dose dependent.

DISCUSSION

In the current study to evaluate the genotypes of *C. albicans* strains, a PCR primer pair designed to span the 25S rDNA gene, was employed to discriminate *C. albicans* isolates into genotypes on the basis of their amplified PCR product size. In the present study we found all three genotypes in *C. albicans* and there was a preponderance of genotype A 68.3%, followed by genotype C 21.5% and genotype B 10.3% these results are consistent with those reported by Zhu et al and Girish kumar et al and Bii et al showing that majority of *C. albicans* from clinical sources belongs to genotypes A followed by genotypes C and B respectively.[6,7,11] Zhu et al from China studied 500 *C.albicans* genotypes by PCR based method using primers designed to span the region of the transposable group I intron of the 25S rRNA gene (rDNA).[7] 500 *C. albicans* isolates could be differentiated into three genotypes, 75.0% into genotype A, 19% into genotype B and 6% into genotype C on the basis of the length of the amplified PCR product and they reported susceptibility varies among different genotypes. Kumar G et al from South India analyzed 55 *C. albicans* genotypes by PCR based method using primers designed to span the site of the transposable group I intron of the 25S rRNA gene.[6] The study revealed 70.9% of genotype A, 7.2% genotype B and 21.2% of genotype C isolates and they reported no association between antifungal resistance and genotype. Bii et al reported that PCR amplification of the 25S rDNA gene of

C. albicans from clinical sources blood, sputum, swabs, urine and catheters tips revealed that genotype A was the most predominant genotype (60%) followed by genotypes B (8%), C (16%) and a new genotype named BC (4%) respectively in Kenya.[11] The ratios of genotypes A, B and C, of *C. albicans* varied among the reports, where the ratio of genotype B or C to genotype A of *C. albicans* varied in each group of clinical specimens. These findings may be affected by the kinds of clinical specimens colonized by *C. albicans.* The classification of genotypes using this PCR relies on the existence of group I intron of varying size in the 25S rDNA and group I intron has self-splicing ability which is necessary for the formation of mature25S rRNA.[7] The self-splicing ability can be inhibited by base analogs therefore the strains harboring group I intron will be more susceptible to base analogs, such as flucytosine analogs.[7]

Our results of the antifungal susceptibility testing of *C. albicans* genotypes showed genotype A were more resistant to flucytosine, amphotericin B, caspofungin, fluconazole and voriconazole than either strains of genotype B or genotype C. All genotype B and genotype C isolates were sensitive to amphotericin B, voriconazole, flucytosine and caspofungin. It has also been shown by other researchers that genotype A is more resistant to fluconazole and flucytosine than Genotype B and C. Early reports demonstrated there was correlation between the group I intron in 25S rDNA of *C. albicans* and susceptibility to flurocytocine.[7,12] Zhu et al. from China analyzed 500 *C. albicans* genotypes; antifungal sensitivity test results indicated that strains of genotype A were more resistant to flucytosine than either strains of genotype B or genotype C.[7] But no regularity was observed between the susceptibility of *C. albicans* genotypes to azoles and to amphotericin B. Mohammed et al also showed there were differences among the antifungal susceptibilities of genotype A, B and C to fluocytosine, and fluconazole. [12] Genotype B and C of *C. albicans* were more susceptible to fluocytosine and fluconazole than genotype A, but no differences were found in the antifungal susceptibilities of genotype A, B and C to amphotericin. The genotype A strains were significantly resistant to fluconazole and fluocytosine then genotype B and C. Whereas Kumar G et al. from south India investigated the genetic diversity in addition antifungal susceptibility profiles of 55 *C. albicans* from immunosuppressed patients.[6] PCR method analysis of the transposable intron in the 25S rDNA showed 39 genotype A, 4 genotype B and 12 genotype C isolates. All strains were susceptible to micafungin, 5-flucytosine and miconazole, whereas resistance against amphotericin B (3.6%), fluconazole (3.6%), itraconazole (7.3%) and voriconazole (5.5%) was detected. No association was seen among antifungal resistance and genotype.

CONCLUSION

Many molecular techniques have been used for studying genotypes of *C. albicans* strains. Molecular typing of *C. albicans* is important for understanding its epidemiology and pathogenesis for the development of appropriate infection control strategies. Detection of genotypes of *C. albicans* was done by conventional PCR to identify their association with drug resistance and virulence factors. PCR based method with specific primers to amplify the regions of the transposable group I intron of 25S rDNA gene to differentiate *C. albicans* genotypes is easy and convenient method.

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